Exchange rate filtering of CEST agents using frequency-labeled exchange (FLEX) transfer

Nirbhay Yadav[1,2], Amnon Bar-Shir[1], Craig Jones[1,2], Chien-Yuan Lin[2,3], Jun Hua[1,2], Assaf Gilad[1], Michael McMahon[1,2], and Peter van Zijl[1,2]
[1]*Johns Hopkins University, Baltimore, MD, United States,* [2]*KKI, Baltimore, MD, United States,* [3]*UT Southwestern Medical Center, Dallas, TX, United States*

Introduction: Chemical exchange saturation transfer (CEST) is a new contrast mechanism employing selective saturation pulse(s) to detect exchangeable protons or molecules. Often, it is non-trivial to assign the contrast to specific protons or agents *in vivo* due to the presence of multiple endogenous sources of CEST contrast, the signals of which may overlap due to the proximity of their frequencies (chemical shifts) or due to overlap if signals are broad due to a high exchange rate. A possible solution to increase the specificity would be to weight the CEST images by exchange rate. One approach to accomplish this is by varying the duration or amplitude of the saturation pulse [1], however this can have deleterious consequences (e.g., broadening of the direct water saturation or increase of conventional MT effects when increasing the B_1 field). Recently, Friedman et al. [2] proposed a technique where exchanging protons are detected via frequency-labeled exchange (FLEX) transfer. To improve the specificity of CEST agent detection, we show that FLEX can be used to create exchange rate maps and to weight exchange contrast towards agents with different exchange rates.

Theory: In contrast to saturation transfer, which leads to a decrease in the water signal, FLEX modulates the water intensity depending on the frequency of the exchangeable groups by encoding the chemical shift evolution of exchanging protons within so-called label transfer modules (LTMs, Fig 1). Each LTM consists of (a.) a selective 90$_x$ radiofrequency (RF) which excites protons over a range of frequencies, (b.) a delay (t_{evol}) during which excited protons undergo chemical shift evolution, (c.) a selective 90$_{-x}$ RF pulse which flips the magnetization back to the longitudinal axis, and (d.) another delay (t_{exch}) which allows time for the labeled protons to exchange into the bulk water pool. Each acquisition contains a preparation time (t_{prep}) consisting of a number of LTMs (n) to allow sensitivity enhancement by repeatedly exchanging frequency labeled protons with unlabeled water protons. By performing a series of acquisitions at different evolution times, the water signal, which includes contributions from all labeled protons at multiple frequencies, modulates in the form of a free induction decay (FID) given by eq. 1:

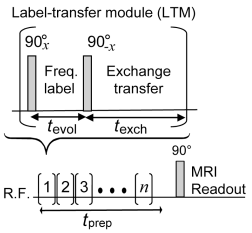

Fig 1. FLEX RF scheme

The FLEX FID modulates with the superposition of frequencies of the individual protons (s), each of which decay at a rate proportional to their exchange rate with water (k_{sw}) plus the transverse relaxation rate (R_2^*) of the exchangeable proton. The modulation frequency is determined by the chemical shift difference between the solute and offset frequency of the FLEX pulses ($\Delta\omega_{so1}$). The magnitude of the contrast is given by eq. 2:

$$\mathrm{FID_{FLEX}} = \sum_{s} \mathrm{PTR_s} \cdot \exp\{-(k_{sw} + R_2^*) \cdot t_{evol}\} \cdot \cos(\triangle\omega_{so1} \cdot t_{evol}) \quad (1)$$

$$\mathrm{PTR_s} = x_s \cdot \lambda_s \cdot (1 - \exp(-k_{sw} \cdot t_{exch})) \cdot \underbrace{\sum_{i=1}^{n} \exp\{-1 + (i-1)/n\}\, t_{prep}/T_{1w}}_{\eta} \quad (2)$$

where x_s is the fractional solute proton concentration, λ_s the excitation efficiency of the selective RF pulses at the solute resonance, and T_{1w} the longitudinal relaxation time constant for water. Since $\Delta\omega_{so1}$ and t_{evol} are known, the FLEX FID can be deconvolved to obtain the components that contribute to the modulation and their (k_{sw}+R_2^*) rate, with R_2^* being negligible compared to k_{sw} for rapidly exchanging protons. Importantly, through the adjustment of t_{exch}, the PTR can be encoded with an exchange rate dependence, which allows the contrast to be weighted for different exchange rates.

Materials and Methods: 20 mM of thymidine was dissolved in PBS and placed in seven 5 mm NMR tubes, with pH adjusted to between 4.3 and 8.1 (see Fig. 2A) in order to vary the exchange rate of the imino NH proton of thymidine. Exchange rates were quantified with the QUESP method and Bloch equation fitting [1], using a 6 s saturation pulse with B_1 field strengths of 1, 3, 5, 7, and 9 μT. The FLEX preparation periods consisted of 500 LTMs, containing 0.2 ms long labeling pulses with o1 = 10 ppm, and t_{evol} was between 0-3 ms in steps of 0.03 ms (dwell time). The FLEX signal was measured as a function of exchange time (chosen to be 3, 5, 8, 12, 18, and 25 ms). Both CEST and FLEX weighted images were acquired on a horizontal bore 11.7 T Bruker Biospec using a RARE pulse sequence.

Table 1. Exchange rates of the thymidine imino NH protons.

pH	4.3	5.3	6.2	6.9	7.2	7.5	8.1
k_{sw} **(× 10^3 s^{-1})**	0.3	0.4	1.1	2.7	3.7	5.2	8.1

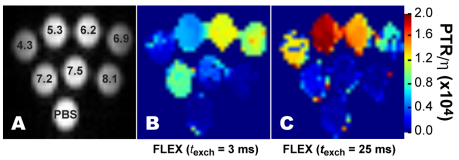

Fig 2. A: Tube arrangement of thymidine phantoms. B: Contrast weighted towards k_{sw}~1-5×10^3 s^{-1} and C: k_{sw}<1×10^3 s^{-1}.

Results and Discussion: Thymidine is a diaCEST contrast agent that can be used to assess the activity of the herpes simplex virus thymidine kinase (HSV-tk) reporter gene. The exchange rate of the imino NH proton of thymidine as a function of pH measured by the QUESP experiment is given in Table 1. Figs. 2B-C show how the contrast from the agent can be weighted towards separate exchange rates. Fig 2B, obtained with t_{exch} = 3 ms, shows moderate contrast from the tubes with $k_{sw} \sim 1\text{-}5 \times 10^3$ s^{-1} and low contrast from k_{sw} < 1000 s^{-1}. When t_{exch} is increased to 25 ms (Fig. 2C) from tubes with k_{sw} < 1000 s^{-1} is significantly enhanced. Fig. 3 shows the buildup of exchange effect for different t_{exch} where the maximum contrast for k_{sw} > 1000 s^{-1} is reached very early because 3 ms, which is much greater than the average bound lifetime of these protons, is enough time for these protons to completely exchange into the water pool. The slower exchanging protons in the solutions at pH 4.3 and 5.3 initially show low contrast but increase significantly once they are given more time to exchange into the water pool. At longer exchange times, the contrast from the rapidly exchanging protons begins to drop, which is likely caused by labeled protons exchanging back from the water pool into the solute pool. The low PTR in the pH 8.1 tube is likely caused by the protons exchanging before they can be labeled by the selective FLEX prepulses. The PTR from these protons, and those with much higher k_{sw}, can be increased by using shorter FLEX labeling pulses [3] however, in this study, this would have resulted in lower signal from most of the other tubes. The data in Figs. 2-3 was normalized by η to remove the effects of longitudinal relaxation (see Eq. (2)).

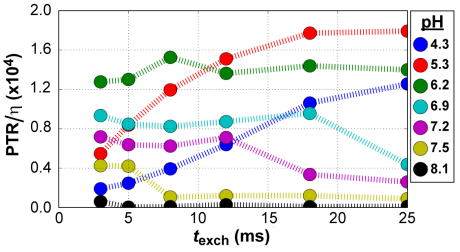

Fig 3. Variation in the FLEX exchange contrast for different t_{exch}.

Conclusion: This study shows that it is possible to weight proton exchange contrast based on exchange rate. This opens up the possibility of filtering CEST contrast based on exchange rate distinguishing between protons with different exchange rates in vivo.

References: 1. McMahon et al. *Magn Reson Med* **55**: 836 (2006). 2. Friedman et al. *J Am Chem Soc* **132**: 1813 (2010). 3. Lin et al. *Proc. 19th ISMRM*, 709 (2011).
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